Effect of \(N\)-methyl-\(N'\)-nitro-\(N\)-nitrosoguanidine on carbohydrate profiles of non-metaplastic rat gastric mucosa

Hans-Peter Sinn¹, Antonio de Oliveira Neto¹, Thomas Lehnert², and Eleanor E. Deschner³

¹ Department of Pathology, University of Heidelberg;
² Department of Surgery, University of Heidelberg, Im Neuenheimer Feld 220, D-6900 Heidelberg, Federal Republic of Germany
³ Memorial Sloan-Kettering Cancer Center, New York, N.Y., USA

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Summary. The effect of \(N\)-methyl-\(N'\)-nitro-\(N\)-nitrosoguanidine (MNNG) on the mucin phenotype of non-metaplastic gastric mucosa in the rat was studied histochemically. Animals were exposed to MNNG in drinking water (83 mg/l) for 12 weeks. Carcinogen treatment was then discontinued and the animals (27 in the treatment group and 25 in the control group) were examined after another 44 weeks. Glycosylation was analysed with histochemical stains for sialomucins and sulphomucins and with peroxidase-conjugated lectins (GS-II, SBA, DBA, UEA-I, and WGA). Sialo- and sulphomucins remained quantitatively unchanged, only a slight increase of acid mucins in the antral glands was observed. The analysis of the lectin binding patterns, however, revealed a significant increase for WGA-binding glycoproteins in the surface mucous cells and gastric pits, while DBA binding was significantly decreased (\(P<0.05\)). GS-II lectin bound specifically to the proliferative compartment in the gastric fundus, consisting of mucous neck cells, and was significantly increased after MNNG treatment. No specific alterations were detected in lectin binding to parietal or chief cells. It is concluded, therefore, that treatment of gastric mucosa with MNNG alters the glycoprotein metabolism before intestinal metaplasia can be observed.

Key words: MNNG – Carcinogenesis – Gastric mucosa – Rats – Lectins – Glycoproteins

Introduction

Gastric mucus is essential for the maintenance of the functional and morphological integrity of gastric mucosa (Rees and Turnberg 1982). Characteristic changes in the mucin content in precancerous lesions of the human stomach have previously been demonstrated using histochemical techniques (Filipe and Jass 1980) and also in lectin binding studies (Filipe and Jass 1986). With the aid of lectins, a further classification of mucins into classes with distinct lectin-binding characteristics is possible in the human (Macartney 1986; Fischer et al. 1984; Kuhlmann et al. 1983) and rat (Suganuma et al. 1985; Kuhlmann and Peschke 1984) stomach mucosa. A number of well-defined and highly purified lectins bind specifically to terminal oligosaccharide structures of epithelial mucins, yielding specific reaction patterns. While the chronic gastritis – intestinal metaplasia – carcinoma sequence has been well characterized in humans (Filipe and Jass 1986; Nardelli et al. 1983; Jass et al. 1984), little information is available on the histogenesis of gastric carcinomas arising without pre-existing gastric mucosal atrophy (Ghandur-Mnaymneh et al. 1988). In the rat, MNNG (\(N\)-methyl-\(N'\)-nitro-\(N\)-nitrosoguanidine) has been widely used to induce adenocarcinomas in the glandular stomach (recently reviewed by Saito 1986). Therefore we used this animal model to examine the long-term effect of MNNG on the mucin secretion of stomach mucosa in the absence of intestinal metaplasia or focal adenomatous changes.

Materials and methods

Animals. A total of 52 male Wistar rats weighing 100 g (supplied by Charles Rivers Laboratories, Kingston, N.Y.) were fed a commercial diet (Purina, Ralston, St. Louis, Mo.) and had free access to drinking water. MNNG (CAS 70-25-7, Aldrich Chemicals, Milwaukee, Wis.) at a concentration of 83 mg/l was given in the drinking water. Fresh solutions were prepared daily and bottles were covered with foil to prevent deterioration of the carcinogen by light. A group of 27 animals were treated with the carcinogen for 12 weeks, while 25 animals received plain tap water. After a period of 44 weeks all animals were killed, i.e. in the 56th week of the experiment. Stomachs were removed, rinsed, pinned out on cardboard and fixed with 10% neutral buffered formalin. Defined areas of the antrum and fundus were sectioned for histological examination (Lehnert et al. 1988).

Histology. Histological sections were stained with haematoxylin and eosin, periodic acid/Schiff, alcian blue, pH 2.5 and 1.0, and high iron diamine (Spicer 1965) (no combined stains or counterstain). For lectin histochemistry, the deparaffinized sections were incubated with peroxidase-conjugated lectins GS-II, SBA, DBA, UEA-I and WGA (E-Y lab-

Abbreviation: MNNG, \(N\)-methyl-\(N'\)-nitro-nitrosoguanidine

Correspondence to: H.-P. Sinn
Table 1. Specificities of lectins used as markers for gastric glycoproteins and inhibiting sugars (modified after Filipe 1989)

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Source</th>
<th>Sugar specificity</th>
<th>Inhibiting oligosaccharide</th>
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<tbody>
<tr>
<td>GS-II</td>
<td><em>Griffonia simplicifolia</em></td>
<td>β-D-GlcNAc</td>
<td>α-GlcNAc</td>
</tr>
<tr>
<td>DBA</td>
<td><em>Dolichos biflorus</em></td>
<td>GalNAc-α1GalNAc</td>
<td>α-GalNAc</td>
</tr>
<tr>
<td>SBA</td>
<td>Soybean (Glycine max)</td>
<td>GalNAcα1-3GalNAc</td>
<td>α- or β-GalNAc</td>
</tr>
<tr>
<td>UEA-I</td>
<td><em>Ulex europaeus</em> I</td>
<td>t-Fucα1-2Galβ1-4GlcNAc</td>
<td>α-Fuc</td>
</tr>
<tr>
<td>WGA</td>
<td>Wheat germ (Triticum vulgaris)</td>
<td>N, N', N''β-Glctriose</td>
<td>β-GlcNAc</td>
</tr>
</tbody>
</table>

oratories, San Mateo, Calif.) at 1:50 dilution in 0.01 M phosphate-buffered saline (PBS, pH 7.4) for 1 h (see Table 1 for specificities). After three washes in PBS, the sections were stained with aminoethylcarbazole as colour substrate and mounted without counterstaining. Control sections were incubated with specific inhibiting sugars (Table 1) at 0.5 M concentration for 1 h after lectin incubations and then counterstained. Cases with mucosal autolysis were excluded from further analysis.

*Image analysis.* For quantitative analysis of the lectin and mucin staining patterns, sections were examined at 100 x magnification and analysed with the ImageAnalyst software system run on an Apple Ix computer with a high-resolution monitor. One representative histological area of fundus and of antrum mucosa was selected from each stomach for measurement. Histological fields were selected to include strictly perpendicularly sectioned mucosa away from any focal hyperplastic or dystplastic area. Within these histological fields the percentage of positive-staining mucosa was determined using grey-scale differentiation of the stained cells against the unstained background. Only mucosal cells were measured and positive-staining material covering the mucosal surface was excluded.

*Statistical analysis.* Values obtained by image analysis measurements, were checked visually for unimodal distribution. Mean values were compared with a two-tailed t-test; P values of 0.05 or less were considered significant.

**Results**

*Mucin histochemistry*

In the fundic mucosa of both the treatment and control groups, surface mucous cells and gastric pits stained slightly positive for sialomucins (alcian blue at pH 1.0 and at pH 2.5). Sulphomucins were detected in foveolar epithelial cells using the high-iron diamine stain. No change in reaction patterns was seen after MNNG treatment and image analysis also revealed no quantitative differences (Fig.1A). Similar results were obtained in the gastric antrum.

*Lectin binding*

Surface mucous cells and gastric pits were positive for the DBA and SBA lectins in the fundic mucosa (Fig. 2C, D), and showed weak or inconsistent binding of the WGA and UEA-